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Nutritional status of fish meal processed by traditional cooking methods of Eastern Sri Lankan

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Abstract
Fish contains high level of omega-3 polyunsaturated fatty acids which are very important for prevention non-communicable diseases. Fish is commonly consumed in the form of fish curry prepared with coconut cream or fried in coconut oil in Sri Lanka and South Asia. This research was carried out to understand the nutritional status of fish meal, particularly omega-3 fatty acids content, which is obtained by the consumers. The fish meal was prepared as per the traditional methods, curried fish and fried fish. The lipid profiles was assessed in raw fish, curried and fried fish. The content of Omega-3 fatty acids and other essential fatty acids was analyzed by the biochemical methods (AOAC). The atherogenicity index (AI) and thrombogenicity (TI) indices were calculated from the fatty acid profile. Ratio of hypocholesterolemia and hypercholesterolemic fatty acids was assessed. Results showed that fatty acid compositions changed significantly during fish preparation by either cooking as curry or frying in coconut oil, and resulted in lower nutritive values than raw fish. Particularly, the healthy lipids EPA and DHA were lost during fish preparation. The thrombogenic index was significantly increased in fried fish, adding to the evidence that consuming fried fish is less beneficial to human health than eating fish cooked in coconut cream.

Keywords: omega-3 fatty acids, nutrition, fish, biochemistry, lipid profile

Introduction

Fish play an important role in human nutrition as they contain omega-3 polyunsaturated fatty acids (PUFAs), such as eicosapentaenoic acid (EPA; C20:5n-3) and docosahexaenoic acid (DHA; C22:6n-3) [1], which are beneficial for health. These fatty acids (FAs) play a role in prevention of atherosclerosis, heart attack, coronary heart diseases [2] and cancer [3]. Fish is commonly consumed in the form of fish curry prepared with coconut cream or fried in coconut oil in Sri Lanka and South Asia. However, fish is usually cooked in different ways for consumption around the world, except the Far East countries where raw fish is popular. Cooking (boiling, baking, roasting, grilling, and frying) improves the microbiological safety of food by neutralizing pathogenic microorganisms and enhances digestibility and the bioavailability of nutrients [1]. During cooking, chemical and physical reactions occur that either improves or impairs nutritional value of food; content of thermolabile compounds and fat-soluble vitamins or PUFAs are often reduced [4]. The effect of processing on the stability of omega-3 PUFAs in fish [5] has been reported. Culinary processing renders them prone to lipid oxidation and loss of long chain omega-3 PUFAs, which reduces the nutritive value of fish [6]. Fat and FA content of fish is extremely variable, even within species, depending upon different abiotic and biotic factors, such as the species, capture area, temperature, and fishing season, pH, salinity, and reproductive cycles [7]. Furthermore, the content of saturated FAs (SFAs) and monounsaturated FAs (MUFAs) changes with cooking and frying, and their amounts depend primarily on the total lipid content, which depends on numerous biological factors [8]. The MUFAs are dominated by oleic acid C18:1n9, followed by the lower concentrations of C16:1n7. In carnivorous species, DHA are more abundant than EPA, and marine fish have more PUFAs than fresh water fish [8].

Fried foods are popular because of their taste, flavor, aroma, crispness, and golden-brown color [9]. Boiling can result in losses of minerals and vitamins by leaching [9]. Consumption of fried fish is also associated with harmful health effects such as systolic dysfunction and potential coronary atherosclerosis [10]. The changes in lipid content and FA composition in fried foods are higher than those in foods prepared by other cooking processes [11].

Material and methods

1. Location and sample collection

The most commonly consumed marine fish in Sri Lanka were collected from the urban and local markets of the Batticaloa district of Eastern Sri Lanka and immediately brought to the cafeteria wrapped in polythene bags. Fish were cleaned and muscle tissue was removed from the mid abdomen of the body. This tissue was washed with cold water and used for cooking and frying.

2. Materials for traditional Sri Lankan fish curry

1. Fish - 2kg
2. Tomatoes- Chopped (2)
3. Onion- 1 large (sliced)
4. Garlic - 10 cloves (sliced)
5. Curry leaves – 2 sprigs
6. 6. Green chilli - 3
7. Chilli powder- 5 TSP
8. Turmeric powder- ½ TSP
9. Cumin powder - 1TSP
10. Coriander powder- 3 TSP
11. Coconut milk- (2litres)
12. Salt- 2TSP
13. Tamarind pulp - 3 TSP
14. 14- Water - 4 liters
15. Coconut oil- 5-6 TSP
16. Fenugreek seeds- 3 TSP

17. Cinnamam - 1 stick
18. Mustard seeds - 1TSP
19. Fennel seeds- 2 TSP
20. Big aluminum pan

3. Preparation of traditional fish curry

The fish was cleaned thoroughly, and the muscle (trunk and abdomen) was cut into pieces (65±3 g) and weighed (1.5 kg). The fish pieces were transferred into a bowl and two teaspoons of salt was sprinkled with one teaspoon of turmeric. The dish set aside for 10 minutes. Next, a medium size aluminum pan was kept on medium heat, and coconut oil (50 ml) was added, followed by addition of mustard seeds, fenugreek, fennel seeds, curry leaves, and cinnamon sticks. The mixture was left for 30 seconds. Then, garlic, green chillies, and chopped onion were added for 4–5 minutes until the onion became translucent. Sliced tomatoes were then added and left for 2 minutes, followed by addition of chilli powder, coriander powder, cumin powder, and salt. After stirring well, 2 liters of diluted coconut cream and tamarind pulp were poured into the mixture and cooked for 4 minutes. When the gravy boiled for 5 minutes, the pieces of fish gently were slid into the gravy and left for another 5-10 minutes to cook under low heat with the pan covered. Finally, two cups of concentrated coconut cream (50ml) were added into the boiling fish curry and cooked for another 5 minutes at low heat. Fresh coriander leaves were sprinkled on the curry, and the fish curry was kept away from the flame. (Plate.1).

4. Preparation of fried fish

The fish was cleaned thoroughly, and the scales were removed with the head and tail cut-off. The trunk was weighed, and the fish was transferred into the bowl, two teaspoons of salt and one teaspoon of turmeric were sprinkled over it and set aside to marinate for 10 minutes. Coconut oil (Turkey, Sri Lanka) was used for frying. One liter of coconut oil was added into the frying pan and boiled for 5 minutes. At about 92±02 °C, marinated fish were slid into the oil for frying. The fish were stirred for 20 minutes until they were golden brown.

5. Determination of fatty acid composition of cooked and fried fish

5.1 Fat Extraction by acid hydrolysis

Muscle tissue from each fish was homogenized using a grinder (Sumeet, Japan). Homogenized muscle samples (3 g) from each fish were weighed in triplicate using an analytical balance (AG204, Mettler, Toledo) and placed in dried conical flasks. Muscle tissue samples were hydrolyzed by adding 8 ml of distilled water and 10 ml of concentrated hydrochloric acid and incubated at 95 °C in a boiling bath for 45 minutes. The samples were cooled and transferred to Mojonnier flasks. Fat was serially extracted three times with 25 ml volumes of petroleum ether: diethyl ether (1:1 v/v). The upper phase, which contained the lipids, was evaporated to dryness and weighed for further analysis.

5.2 Preparation of fatty acid methyl esters (FAMEs)

FAMEs were prepared from muscle samples from lipids extracted from each species. Samples were extracted with petroleum ether/diethyl ether solvent mixture according to modified method of AOAC (948.16). The FAs in the total lipids were esterified into methyl esters by saponification with 0.5 N methanolic NaOH and trans-esterified with 14% BF3 (v/v) in methanol [12].

5.3 Fatty acid analysis using gas liquid chromatography

The FAMEs were analyzed on a Shimadzu-14A model gas chromatograph (GC) (Shimadzu, Japan), equipped with a flame ionization detector (FID) and fitted with a capillary column (Superlcowax-10 polythene glycol; length, 100 m; I. D, 0.25 µm) (Sigma-Aldrich Co LLC, St. Louis, MO). Injector and detector temperatures were 200 °C and 220 °C. The oven program was initially held at 60 °C for 10 min, then increased at a rate of 1 °C/min to 200 °C over 10 minutes, and then held at 200 °C for 55 min (total run time). The flow rate of the N2 carrier gas was 1 °C min1. GC analysis of FAMEs was repeated three times for each sample. FAMEs were identified by comparison of peak retention times to those of standards (NU prep check- SD 461, USA). Samples were run in split mode (50:1). Results were expressed as FID response area, as relative percentages of peak area obtained from GC-FID chromatogram. The results are given as mean ± SD in Table 1.

6. Calculated indices

The atherogenicity index (AI) and thrombogenicity (TI) indices were calculated from the fatty acid profile, as proposed by [13], which relates the profile of FAs with the risk of cardiovascular disorders, using the following equations:

AI = [C12:0 + (C14:0x4) + C16:0] /(Total unsaturated FAs) -1 Eq (1)

Where C12 = the percentage of lauric acid in relation to total fatty acid (TFA); C14 = the percentage of myristic acid in relation to TFA; and C16 = the percentage of palmitic acid in relation to TFA.

TI = Σ (C14:0 + C16:0+C18:0)/[(0.5 x cis C18:1 + 0.5 x ΣMSFA+ 0.5x Σ (omega-6) +0.5 x Σ (omega-3) + (omega-3/omega-6)] Eq (2)

Where: MSFA is monounsaturated fatty acid, omega-6 is FAs containing omega-6 and omega-3 is FAs containing omega-3. In addition, the unsaturated/saturated (USAT/SAT), n-3/n-6 ratio and Σ EPA+DHA were calculated using the fatty acid profile in extracting lipid from muscle.

Ratio between hypocholesterolaemia and hypercholesterolemic fatty acids was calculated using the equation below.

(HH)=(C18:1n-9+C18:2n-6+C20:4n-6+C18:3n-3-+C20:5n-3+C22:5n-3+C22:6n-6)/(C14:0+C16:0)

7. Statistical analysis

Data were analysed statistically using one-way analysis of variance (ANOVA), p <.05, using SPSS 10.0. Means were compared using Tukey's multiple comparison test or Student's t-test. SAS 9.1.3. was also used to compare the data whether it was significantly different one treatment to other.

Table 1: Fatty acid Composition of raw, curried and fried fish dish Sri Lankan

	***Leiognathus bindus* (Pony Fish)**			***Mugil cephalus* (Mullet)**			***Sphyraenea jello* (Baracuda)**			***Dussumieria acuta* (herrings)**		
Fatty acid	Raw fish	Fried fish	Curried fish	Raw fish	Fried fish	Curried fish	Raw fish	Fried fish	Curried fish	Raw fish	Fried fish	Curried fish
C12:0	0.12±0.51[b]	0.37±0.01[b]	10.53±0.20[a]	0.02±0.00[b]	0.34±0.00[b]	17.17±1.21[a]	0.58±0.06[b]	0.74±0.09[b]	8.12±0.77[a]	0.22±0.01[b]	0.29±0.02[b]	36.73±2.26[a]
C14:0	4.73±0.05[b]	1.25±0.03[c]	8.14±0.27[a]	2.98±0.10[b]	2.28±0.01[c]	7.68±0.38[a]	6.54±0.12[a]	1.30±0.06[c]	5.98±0.38[b]	3.76±0.01[b]	2.04±0.07[c]	13.76±1.68[b]
C15:0	0.92±0.03[c]	0.09±0.01[b]	1.00±0.02[a]	2.33±0.07[a]	0.20±0.01[c]	0.38±0.02[b]	5.40±1.88[a]	0.10±0.00[b]	0.65±0.03[c]	3.74±0.02[a]	0.11±0.01[b]	0.13±0.01[b]
C16:0ISO	5.84±2.97	2.01±0.00	6.11±0.01	0.40±0.06	0.04±0.02	0.04±0.02	1.56±0.17[a]	0.06±0.00[b]	0.07±0.00[c]	1.74±0.01[a]	0.02±0.00[c]	0.25±0.00[b]
C16:0	25.21±0.11[b]	36.36±0.27[a]	23.39±0.90[b]	17.93±0.55[b]	35.41±0.41[a]	14.29±0.45[c]	24.30±1.47[b]	32.00±0.59[a]	17.14±0.69[c]	24.30±1.47[b]	31.94±0.41[a]	11.51±0.77[c]
C18:0	9.38±4.69[a]	4.42±0.03[b]	9.51±0.29[a]	11.08±0.30[b]	4.37±0.07[a]	5.17±0.14[b]	5.54±0.19[b]	4.03±0.08[c]	6.94±0.12[a]	9.66±0.01[a]	3.93±0.02[b]	3.43±0.23[c]
C20:0	0.49±0.25[a]	0.34±0.01[b]	0.38±0.22[c]	0.17±0.01[b]	0.417±0.23[a]	0.23±0.01[b]	0.79±0.00[a]	0.37±0.02[b]	0.35±0.04[b]	2.66±0.20[a]	0.32±0.00[b]	0.01±0.01[c]
C16:1n7	5.84±0.35[a]	2.01±0.02[b]	6.11±0.46[a]	6.67±0.19[a]	3.59±0.03[b]	1.94±0.03[c]	7.06±0.39[a]	0.86±0.04c	2.98±0.10[c]	3.66±0.01[b]	5.99±0.08[a]	0.78±0.07[c]
C16:`1n5	0.21±0.02[c]	0.04±0.01[b]	0.59±0.07[a]	1.23±0.02[a]	0.17±0.02[b]	0.10±0.06[c]	0.14±0.02a	0.07±0.10[b]	0.12±0.01[a]	0.29±0.00[a]	0.20±0.00[b]	0.12±0.07[b]
C181n9	9.24±0.20[b]	36.59±0.16[a]	9.31±0.28[b]	0.92±0.00[b]	34.14±0.64[a]	13.19±0.66[b]	14.37±2.04[b]	37.75±0.94[a]	13.01±0.08[b]	5.54±0.00[c]	33.40±0.50[a]	7.02±0.47[b]
C18:1n7	0.13±0.11[b]	0.28±0.01[b]	2.76±0.11[a]	5.75±0.14	0.15±0.10	0.43±0.02	0.90±0.00[a]	0.02±0.01[b]	0.96±0.00[a]	1.35±0.17[a]	0.74±0.01[b]	0.66±0.02[b]
C20:1n9	0.52±0.26[b]	0.21±0.01[c]	1.42±0.12[a]	0.04±0.01[c]	0.12±0.03[b]	0.33±0.08[a]	0.19±0.04[b]	0.36±0.01[a]	0.31±0.13[a]	0.42±0.01[a]	0.19±0.01[c]	0.21±0.01[b]
C22:1n9	0.96±0.52[a]	0.14±0.09[b]	0.29±0.02[b]	6.13±0.20[a]	0.18±0.12[b]	0.31±0.30[b]	0.24±0.00[b]	0.26±0.10[b]	0.64±0.13[a]	2.42±0.01[a]	0.46±0.12[b]	0.51±0.01[b]
C18:2n6	1.06±0.14[b]	8.89±0.03[a]	1.57±0.45[b]	0.61±0.08[c]	7.89±0.07[b]	12.08±0.31[a]	0.31±0.01[c]	11.23±0.39[a]	9.46±0.20[b]	0.03±0.00[c]	11.77±0.10[a]	2.78±0.18[b]
C18:3n6	0.08±0.05	0.28±0.01	ND	0.81±0.03[a]	0.22±0.04[b]	0.58±0.02[c]	0.18±0.01[b]	0.55±0.09[c]	1.24±0.00[c]	0.29±0.01[c]	0.95±0.01[b]	1.11±0.00[a]
C18:3n3	0.46±0.24[a]	0.09±0.05[c]	0.21±0.03[b]	1.10±0.03[a]	0.26±0.02[c]	0.74±0.01[b]	0.48±0.01[b]	0.19±0.11[c]	0.70±0.01[a]	0.34±0.01[a]	0.13±0.01[b]	0.12±0.04[b]
C18:4n3	0.42±0.23	0.06±0.03	0.23±0.03	0.33±0.00[a]	0.24±0.00[a]	0.41±0.22[a]	1.25±0.02[a]	0.07±0.03[c]	0.49±0.05[b]	0.02±0.00[b]	0.08±0.04[b]	0.40±0.20[a]
C20:2n6	0.22±0.13	0.00±0.00	0.40±0.01	0.22±0.01	0.00±0.00	0.30±0.23	0.13±0.01[b]	0.03±0.02[c]	0.13±0.00[b]	0.25±0.02[a]	0.03±0.02[a]	0.25±0.68[a]
C20:3n6	0.16±0.09[a]	0.00±0.00	0.20±0.02[b]	0.31±0.01[a]	0.06±0.01[b]	0.08±0.08[b]	0.18±0.01[a]	0.65±0.55[a]	0.08±0.02[c]	2.72±0.00[a]	0.04±0.01c	1.77±0.27[c]
C20:4n6	2.82±1.55[a]	0.29±0.01[b]	2.69±0.03[a]	3.33±0.06[a]	0.89±0.19[c]	1.51±0.12[b]	0.04±0.01[c]	1.02±0.53[b]	1.88±0.02[a]	5.41±0.01[a]	0.52±0.01[b]	0.82±0.46[b]
C20:3n3	0.13±0.06[a]	0.06±0.01[a]	0.15±0.03[a]	1.40±0.20[a]	0.11±0.05[b]	0.23±0.11[b]	0.06±0.02[a]	0.24±0.36[a]	0.08±0.04[a]	0.02±0.00[b]	0.13±0.01[b]	0.42±0.20[a]
C20:4n3	0.33±0.17[a]	0.06±0.02[a]	0.38±0.06[a]	0.43±0.22[a]	0.22±0.25[a]	0.24±0.16[a]	0.45±0.11[a]	0.20±0.28[a]	0.29±0.01[a]	0.01±0.01[b]	0.10±0.05[b]	4.38±0.47[a]
C20:5n3	3.42±1.83[b]	0.57±0.03[c]	5.74±0.03[a]	2.59±0.09[b]	1.74±0.11[a]	1.84±0.24[a]	7.22±0.38[a]	1.03±0.16[c]	3.73±0.08[b]	0.65±0.01[b]	1.87±0.37[a]	1.46±0.16[a]
C21:5n3	0.15±0.08	0.06±0.06	0.14±0.09	1.236±0.01[a]	0.08±0.01[b]	0.10±0.05[b]	1.53±0.54[a]	0.05±0.00[b]	0.13±0.08[b]	0.06±0.00[a]	0.11±0.09[a]	0.12±0.00[a]
C22:4n6	0.91±0.48[b]	0.18±0.22[c]	1.65±0.04[a]	0.133±0.01[b]	0.09±0.01[c]	0.24±0.02[a]	0.68±0.01[a]	0.05±0.03[c]	0.36±0.12[b]	0.01±0.00[b]	0.06±0.04[a]	0.07±0.00[a]
C22:5n6	1.52±0.83[a]	0.19±0.06[c]	0.52±0.03[b]	1.143±0.01[a]	0.23±0.01[c]	0.88±0.02[b]	2.14±0.02[a]	0.21±0.01[c]	1.63±0.12[b]	2.13±0.01[a]	0.16±0.08[b]	0.21±0.03[b]
C22:5n3	1.72±0.00[a]	0.19±0.05[c]	1.90±0.17[a]	2.20±0.07[a]	0.78±0.07[b]	0.73±0.03[b]	2.430.00[a]	0.39±0.14[c]	0.83±0.03[b]	0.94±0.01[a]	0.27±0.06[b]	0.15±0.09[b]
C22:6n3	17.03±9.46[a]	1.35±0.12[c]	2.52±0.16[b]	6.126±0.24[b]	1.96±0.26[c]	2.91±0.19[a]	13.38±0.55[b]	2.99±0.06[c]	2.20±0.06[a]	24.21±0.02[a]	1.28±0.01[c]	2.43±0.07[b]

ND- not detected, Data presented mean ±SD. [a, b, c], same letter not significant and different letters statistically significant. Table1. Continues

	***Rasterlliger kanagurata* (Mackeral)**			***Katsuwonus pelamis* (Tuna)**			***Selar crumenophtthalamus* (Scad)**			***Chirocentrus dorab* (Wolf herring)**		
FattAcid	Rawfish	Fried fish	Curried fish	Raw fish	Fried fish	Curried fish	Raw fish	Fried fish	Curried fish	Raw fish	Fried fish	Curried fish
C12:0	1.20±0.07[c]	3.47±0.12[b]	4.80±0.20[a]	0.08±0.01[a]	0.3±0.00[b]	8.9±0.52[b]	0.30±0.06[c]	0.74±0.04[a]	0.39±0.03[b]	0.30 ±0.00[c]	0.74 ±0.05[a]	0.39 ±0.48[b]
C14:0	1.00±0.01[b]	3.59±0.10[a]	3.73±0.12[a]	1.13±0.09[a]	1.05±0.01[b]	5.45±0.30[b]	4.14±0.75[a]	1.19±0.10[b]	1.08±0.04[b]	5.34 ±0.00[a]	1.66 ±0.00[b]	5.44±0.25[a]
C15:0	7.00±0.01[a]	0.22±0.01[b]	0.53±0.02[c]	0.54±0.04[a]	0.13±0.01[b]	0.41±0.02[b]	1.06±0.21[a]	0.20±0.15[b]	0.13±0.00[b]	5.34 ±0.01[a]	0.24 ±0.01[c]	0.41 ±0.01[b]
C16:0ISO	3.90±0.00[a]	0.02±0.00[b]	0.05±0.01[b]	0.04±0.01[a]	0.05±0.01[c]	0.18±0.01[b]	0.15±0.06[a]	0.03±0.00[b]	0.06±0.00[b]	0.30 ±0.00[c]	0.08 ±0.01[a]	0.06 ±0.00[b]
C16:0	3.60±0.02[c]	33.79±0.56[a]	13.81±0.42[b]	18.46±1.25[a]	32.04±0.15[c]	14.35±083[b]	25.16±1.18[c]	30.86±2.79[b]	35.33±0.41[a]	25.78 ±0.13[b]	32.57 ±0.38[a]	17.71±0.58[c]
C18:0	6.00±0.01[b]	5.10±0.04[c]	7.72±0.26[a]	7.74±5.90[a]	5.21±0.03[a]	5.48±0.34[a]	11.19±0.68[a]	3.76±0.42[c]	4.97±0.03[b]	8.92±0.01[a]	4.85±0.07[c]	5.29±0.05[b]
C20:0	1.70±0.37[a]	0.37±0.01[c]	0.42±0.02[b]	0.22±0.03[a]	0.28±0.03[b]	0.29±0.01[a]	0.70±0.26[a]	0.37±0.04[b]	0.39±0.01[b]	0.49±0.01[a]	0.35±0.01[c]	0.43±0.01[b]
C16:1n7	1.10±0.01[c]	2.55±0.02[b]	2.58±0.07[a]	3.70±0.20[a]	3.95±0.29[b]	3.06±0.19[a]	3.93±0.53[a]	0.56±0.12[c]	1.88±0.01[b]	1.24 ±0.01[c]	4.05 ±0.09[b]	4.25 ±0.07[a]
C16:`1n5	3.60±0.01[a]	0.08±0.02[b]	0.11±0.01[b]	0.26±0.10[a]	0.10±0.02[b]	0.15±0.01[ab]	0.05±0.01[b]	0.12±0.00[a]	0.04±0.00[c]	0.03 ± 0.01[c]	0.13 ±0.01[b]	0.19±0.03[a]
C181n9	5.20±0.01[c]	31.78±0.35[a]	14.18±0.44[b]	11.12±1.04[a]	32.22±0.21[c]	17.28±1.05[b]	13.33±2.45[c]	36.60±3.00[b]	37.38±0.08[a]	10.40±0.01[c]	33.06±0.46[a]	14.44±0.15[b]
C18:1n7	1.21±0.01[a]	0.22±0.01[c]	0.99±0.04[b]	1.78±0.08[a]	1.33±0.02[c]	1.00±0.06[b]	1.98±0.10[a]	0.02±0.01[c]	0.43±0.02[b]	1.52±0.01[a]	0.89±0.02[b]	0.31±0.02[c]

C20:1n9	2.20±0.01^{a}	0.27±0.02^{b}	0.24±0.01^{c}	0.81±0.06^{a}	0.26±0.03^{b}	0.18±0.03^{b}	0.51±0.01^{a}	0.21±0.02^{c}	0.30±0.00^{c}	0.65±0.20^{a}	0.31±0.09^{b}	0.22±0.01^{b}
C22:1n9	2.20±0.01^{a}	0.27±0.02^{b}	0.24±0.01^{c}	0.53±0.17^{a}	0.55±0.12^{a}	0.48±0.09^{a}	0.51±0.06^{a}	0.14±0.01^{b}	0.18±0.02^{a}	0.02±0.00^{c}	0.42±0.05^{b}	0.96±0.20^{a}
C18:2n6	1.25±0.04^{c}	6.83±0.08^{b}	12.92±0.41^{a}	1.42±0.07^{a}	7.13±0.08^{c}	12.87±0.79^{c}	2.56±0.67^{c}	11.45±0.93^{a}	8.68±0.02^{b}	0.56±0.05^{c}	7.39±0.11^{b}	16.14±0.22^{a}
C18:3n6	6.60±0.02^{a}	0.22±0.02^{c}	0.74±0.45^{b}	0.53±0.02^{a}	0.54±0.02^{b}	1.07±0.23^{b}	0.03±0.00^{b}	0.03±0.00^{b}	0.34±0.01^{a}	0.15±0.01^{b}	0.59±0.01^{a}	0.07±0.02^{c}
C18:3n3	1.20±0.01^{a}	0.25±0.02^{c}	0.72±0.04^{b}	0.12±0.02^{a}	0.08±0.02^{b}	0.79±0.05^{b}	0.39±0.03^{a}	0.13±0.02^{b}	0.15±0.02^{b}	0.13±0.00^{c}	0.19±0.01^{b}	1.22±0.02^{a}
C18:4n3	0.31±0.01^{c}	0.18±0.04^{b}	0.33±0.02^{a}	0.14±0.01^{a}	0.00±0.00^{b}	0.26±0.11^{a}	0.36±0.05^{a}	0.01±0.01^{b}	0.11±0.08^{b}	0.23±0.01^{b}	0.12±0.02^{c}	0.60±0.02^{a}
C20:2n6	4.40±0.01^{a}	0.00±0.00^{c}	0.26±0.01^{b}	0.34±0.03^{a}	0.05±0.02^{c}	0.14±0.01^{b}	0.35±0.03^{a}	0.04±0.00^{b}	;0.03±0.00^{b}	0.17±0.01^{a}	0.04±0.01^{c}	0.09±0.00^{b}
C20:3n6	1.70±0.02^{a}	0.03±0.01^{b}	0.16±0.13^{b}	0.15±0.03^{a}	0.00±0.00^{c}	0.04±0.01^{b}	0.09±0.01^{a}	0.04±0.00^{b}	0.02±0.00^{c}	0.15±0.00^{a}	0.04±0.01^{b}	0.15±0.03^{a}
C20:4n6	2.20±0.01^{a}	0.48±0.01^{c}	1.76±0.08^{b}	4.48±0.28^{a}	1.01±0.01^{b}	1.14±0.08^{b}	1.98±0.27^{a}	0.27±0.06^{b}	0.32±0.00^{b}	2.11±0.11^{a}	0.47±0.06^{b}	2.06±0.07^{a}
C20:3n3	8.00±0.01^{a}	0.08±0.02^{c}	0.35±0.16^{b}	0.67±0.12^{a}	0.04±0.01^{c}	0.28±0.01^{b}	0.09±0.01^{a}	0.10±0.00^{a}	0.08±0.05^{a}	0.33±0.01^{a}	0.17±0.01^{b}	0.25±0.12^{b}
C20:4n3	0.04±0.01^{c}	0.16±0.03^{b}	0.26±0.09^{a}	0.35±0.15^{a}	0.53±0.01^{b}	0.14±0.04^{a}	0.22±0.03^{a}	0.19±0.28^{a}	0.07±0.02^{a}	0.01±0.00^{b}	0.08±0.04^{b}	0.31±0.11^{a}
C20:5n3	5.15±0.01^{a}	1.90±0.34^{c}	3.46±0.51^{b}	4.06±0.30^{a}	1.18±0.30^{c}	2.26±0.09^{b}	3.52±0.47^{a}	0.63±0.03^{b}	0.55±0.05^{b}	5.37±0.02^{a}	0.94±0.72^{c}	3.92±0.16^{b}
C21:5n3	1.20±0.02^{a}	0.07±0.03^{c}	0.10±0.04^{b}	0.13±0.03^{a}	0.00±0.00^{b}	0.03±0.02^{b}	0.07±0.04^{a}	0.07±0.09^{a}	0.03±0.01^{a}	1.01±0.01^{a}	0.03±0.01^{c}	0.35±0.18^{b}
C22:4n6	1.90±0.03^{a}	0.08±0.02^{c}	0.25±0.06^{b}	0.35±0.04^{a}	0.07±0.01^{a}	0.4±0.47^{a}	0.22±0.03^{a}	0.00±0.00^{b}	0.06±0.02^{b}	0.58±0.02^{a}	0.06±0.02^{b}	0.58±0.19^{a}
C22:5n6	2.10±0.03^{a}	0.21±0.00^{b}	2.23±0.10^{a}	3.96±0.24^{a}	0.87±0.02^{c}	1.59±0.02^{b}	2.07±0.27^{a}	0.22±0.07^{b}	0.38±0.02^{b}	2.15±0.01^{a}	0.42±0.31^{c}	1.11±0.19^{b}
C22:5n3	1.65±0.03^{a}	0.32±0.03^{b}	0.76±0.04^{c}	0.67±0.05^{a}	0.21±0.05^{c}	0.49±0.06^{b}	1.38±0.17^{a}	0.31±0.26^{b}	0.16±0.03^{b}	1.24±0.01^{a}	0.27±0.06^{c}	1.08±0.06^{b}
C22:6n3	8.30±0.03^{b}	2.44±0.01^{c}	18.22±0.59^{a}	27.77±1.57^{a}	5.56±0.25^{c}	11.38±0.78^{b}	17.64±2.33^{a}	2.66±0.59^{b}	3.20±0.07^{b}	18.71±0.67^{a}	5.14±0.08^{c}	7.76±0.09^{b}

Table 1: SFA, MUFA, PUFA and lipid indices of the raw, curried and fried fish

Fish Name	Type	SFA	MUFA	PUFA	ω3-PUFA	ω6-PUFA	PUFA/SFA	ω3/ω6	ω6/ω3	SFA/PUFA
Leiognathus bindus	R	40.99±8.19	16.88±4.64	31.22±20.6	23.65±12.08	6.77±8.08	0.76	3.49	0.29	1.31
	F	42.92±0.36	41.27±0.29	12.55±0.81	2.44±0.36	9.81±0.32	0.29	0.25	4.02	3.42
	C	56.49±2.00	21.07±1.25	19.11±0.97	11.27±0.57	7.03±0.57	0.34	1.6	0.62	2.96
Mugil cephalus	R	34.91±1.10	20.94±0.58	15.03±0.69	7.29±0.39	5.28±0.19	0.43	1.38	0.72	2.32
	F	43.13±0.76	38.37±0.95	15.46±0.97	5.38±0.45	9.38±0.33	0.36	0.57	1.74	2.79
	C	50.38±2.76	16.30±1.16	29.15±1.23	13.20±0.41	15.68±0.78	0.58	0.84	1.19	1.73
Rasterlliger kanagurata	R	24.40±0.14	18.30±0.07	51.20±0.22	25.20±0.10	26.10±0.12	2.1	0.96	1.04	0.48
	F	47.48±0.86	35.42±0.60	13.54±0.57	5.40±0.50	7.85±0.50	0.29	0.69	1.45	3.51
	C	32.43±1.11	18.96±0.99	42.29±1.70	23.48±1.70	18.31±1.24	1.3	1.28	0.78	0.77
Chirocentrus dorab	R	30.18±4.77	15.17±2.56	25.25±1.93	10.10±0.49	15.30±0.41	0.84	0.4	2.49	1.2
	F	40.76±0.79	38.77±0.63	16.96±0.38	4.35±0.31	12.61±0.07	0.42	0.76	1.32	2.4
	C	37.10±1.42	20.52±0.46	7.35±0.41	2.35±0.26	4.98±0.15	0.2	0.77	1.3	5.05
Selar crumenophthalamus	R	42.82±3.25	21.78±5.67	31.47±4.69	23.68±3.12	7.28±1.28	0.73	3.25	0.31	1.36
	F	37.36±3.58	37.79±3.23	16.82±2.44	4.09±1.26	11.95±1.06	0.45	0.34	2.93	2.22
	C	42.43±0.53	40.17±0.16	14.50±0.45	4.35±0.34	9.83±0.09	0.34	0.44	2.26	2.93
Katsuwonus pelamis	R	28.22±7.33	18.25±1.64	46.05±3.04	33.91±0.70	11.22±0.70	1.63	2.23	0.31	0.61
	F	39.14±0.25	38.47±0.72	17.54±0.81	7.61±0.15	9.61±0.15	0.44	4.29	0.23	2.28
	C	37.35±2.20	22.16±1.43	33.24±2.01	15.65±1.59	17.26±1.59	0.31	0.23	4.26	3.21
Sphyraenea jello	R	37.87±0.20	24.63±0.08	25.35±0.64	10.00±0.13	15.34±0.51	0.67	3.02	0.33	1.49
	F	38.72±0.88	39.31±1.14	17.19±0.23	5.46±0.12	11.73±0.11	0.45	0.79	1.26	2.21
	C	41.50±2.35	17.18±0.45	11.62±0.36	4.54±0.19	7.08±0.17	0.88	0.91	1.1	1.14
Dussumieria acuta	R	37.87±2.05	11.49±2.93	32.48±1.97	17.24±0.91	6.13±0.67	0.86	2.81	0.36	1.17
	F	38.70±0.52	40.96±0.52	17.92±0.89	3.97±0.65	13.50±0.24	0.46	0.29	3.4	2.16
	C	73.60±5.78	8.58±0.62	14.21±2.25	8.03±0.45	5.55±1.66	0.19	1.45	0.69	5.18

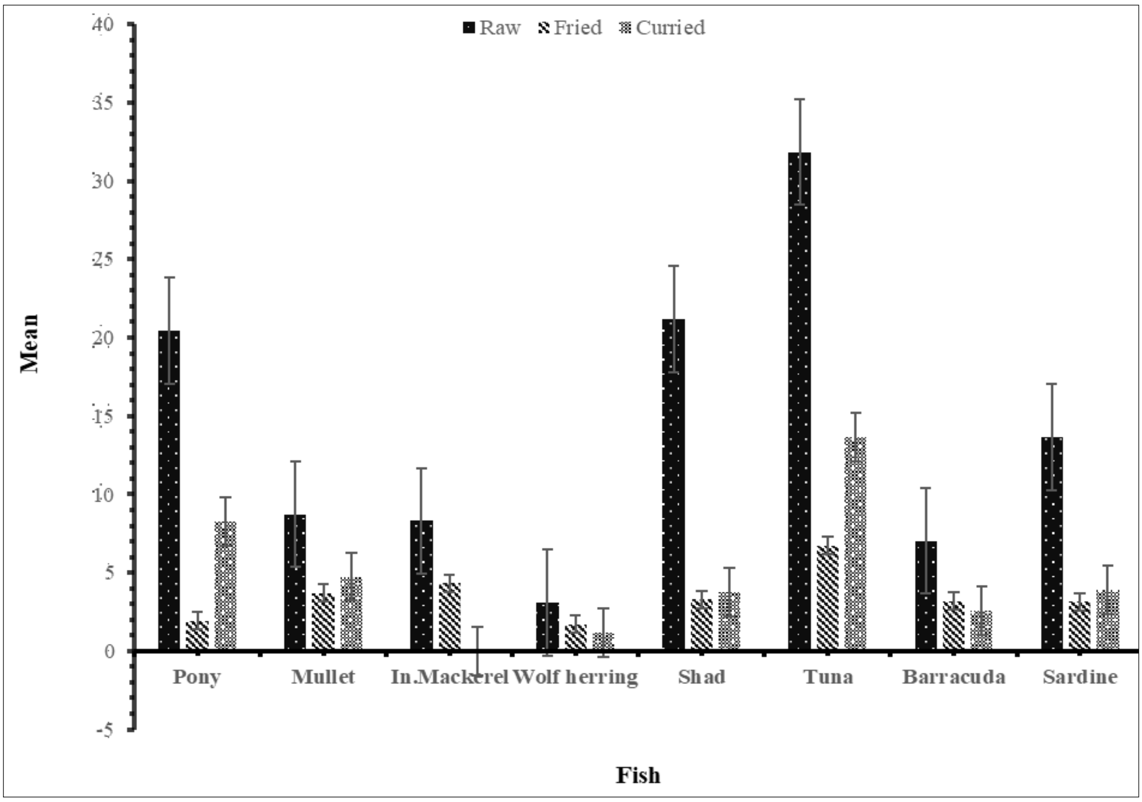


Fig 1: Total EPA and DHA content in raw, fried and curried fish. Data presented as mean± SE

Results and Discussion

1. Fatty acid composition of processed fish

Using eight marine fish species, the fatty acid composition of raw (fresh) fish was compared with that of fish curry made with coconut cream or fish fried in coconut oil. The fatty acid composition changed during the cooking and frying processes, as can be seen in Table 1. Fish fried showed a significant rise in MUFA content in all samples, whereas the omega-3 PUFA content decreased in both fried and fish curry in most samples (Figure 1).

SFA content also showed an increase in both fried and cooked fish in all eight samples (Figure.1). These results are in agreement with those described by [14].

However, reported that the omega-3 fatty acid content did not increase in microwaved samples but decreased in fried samples.

The Sri Lankan style of fish consumption led to a decrease in overall EPA and DHA content (Figure.1). These changes could be interpreted according to the findings of [15], who suggested that two mechanisms may occur during frying: absorption of culinary fat by the fish and leaching of soluble fat molecules out of the food. Therefore, it must be noted that when an exchange of fat between the fish and culinary fat takes place, loss of specific fish fatty acids such as EPA and DHA increase considerably [5]. This would imply that the diffusion of EPA and DHA from trout fillets into culinary fat would result in decreased levels of these fatty acids in the cooked samples [16]. In addition, [15] showed that, during frying, a decrease in total fatty acid content in oily fish changed the fatty acid profile of culinary fat. In contrast, the fatty acid profile in lean fish is similar to that of the culinary fat used.

Officially, the recommended intake of EPA plus DHA for humans by the WHO is 1 g/day [5].

Owing to the popularity of fried and cooked fish, the actual consumption of EPA and DHA decreased in the Sri Lankan community. The results show that the major fatty acids among SFAs and MUFAs in each cooked fish sample were palmitic (C16:0) and oleic (C18:1) acids. In addition, linoleic acid (C18:2) and DHA (C22:6) were the predominant PUFA in both cooked and fried fish (Table.1). The lauric acid (C12:0) content was markedly high in the curry than raw fish because it was made with coconut cream, which contains a high amount of lauric acid (45.98 g/100 g). In contrast, fried fish did not show a high level of lauric acid (Table.1) because it could have been oxidised at the frying stage

PUFA: SFA & Omega- 3: Omega- 6 Ratio

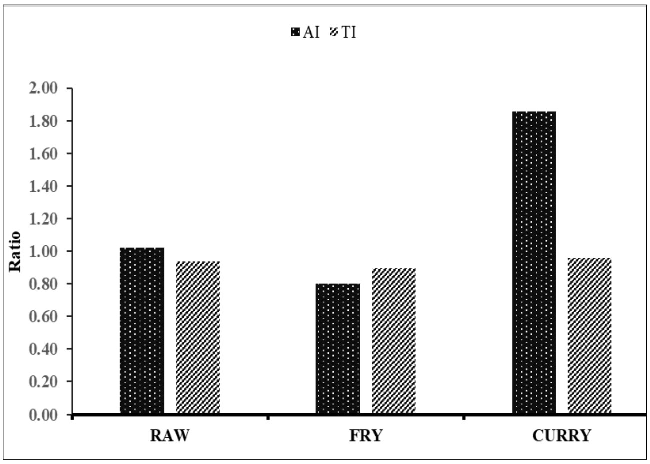
The PUFA: SFA ratio decreased from raw (1.63) to fried (0.31) and curried (0.88) tuna fish, and the omega-3: omega-6 ratio increased from raw (2.23) to fried (4.29) and decreased in curried tuna fish owing to an increase in C20:4n6 and C22:6n6 content and oxidization of PUFA
(Table 2). The ratio of omega-3:omega-6 fatty acids has been suggested as a useful indicator of the relative nutritional value of fish oils. Indeed, it is a key factor for balanced synthesis of composite hormones known as eicosanoids in organisms and in the normal growth and development of the human body [17].

According to current WHO recommendations, the total daily omega-3: omega-6 ratio in the human diet should not be higher than 1.5. The results reveal that tuna fish had the highest omega-3: omega-6 ratio (4.29) and varied, from high to low (from 4.29 to 0.023). The omega-3:omega-6 ratio in tissues of marine fishes varied from 5 to 10, and in fresh water fishes from 1 to 4 [10]. Also, seasonal changes can affect the ratio according to [17]. The traditional method of Sri Lankan cooking of fish (oil fry and curry) showed an omega-3: omega-6 ratio of less than 1 compared to raw fish (Table 2).

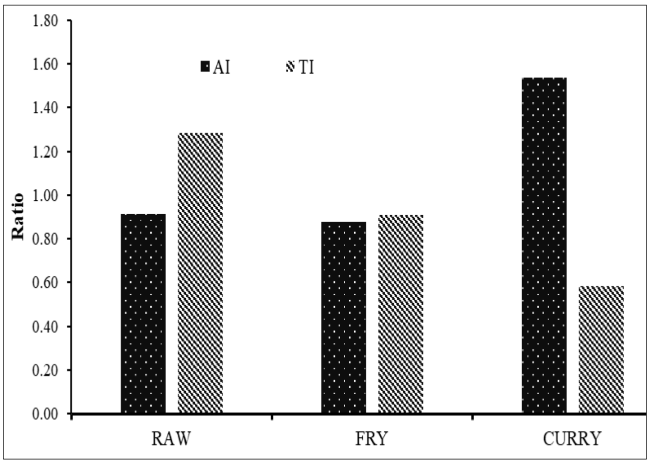
The PUFA:SFA ratio shows that fish oil is a good source of PUFA; the minimum recommended ratio value is 0.45 [12] (Domingo, 2007). The nutritional factor in a sample of raw trout was 0.91, and ranged from 0.87 to 1.64 in cooked trout. This result agrees with [18] where the PUFA: SFA ratio increased significantly in fried and microwaved trout. The observed increase in this ratio is in accordance with other studies [19]. The results presented here show that the PUFA: SFA ratio is less than 1 (Table.2) in most fried and cooked fish samples.

AI and TI of Raw, Fried and Curried fish

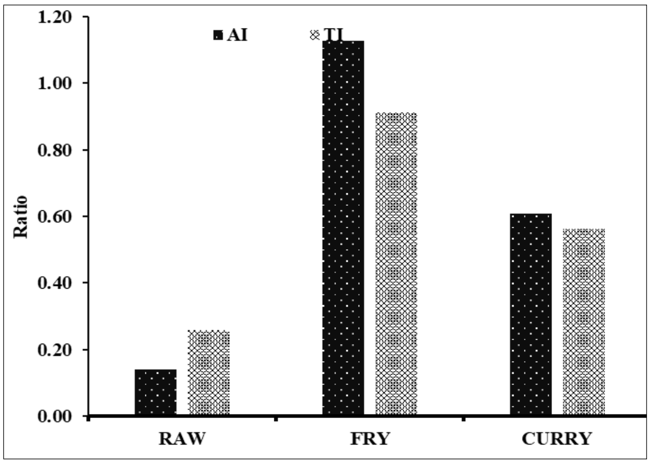
The low values of AI and TI are beneficial to human health according to [20], who suggested that fatty acids prevent the emergence of coronary diseases. Both values were less than 1 in processed fish (Figure.3). Both AI and TI in were low in fresh skipjack tuna, whereas fried fish had higher TI values (>1) owing to the absorbance of coconut oil. In addition, [21] found seasonal variation in AI and TI in wild species.



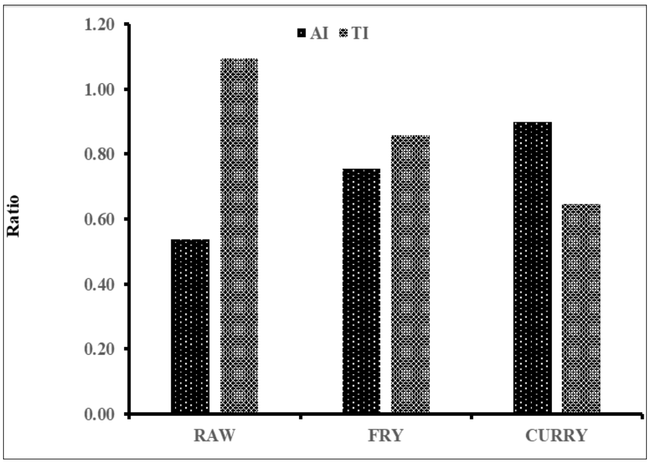
a. Pony fish (*Leionathus bindus*)



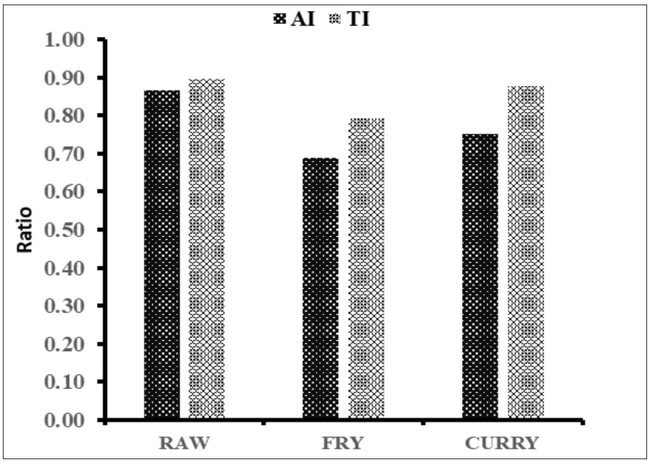
b. Mullet (*Mugil cephalus*)



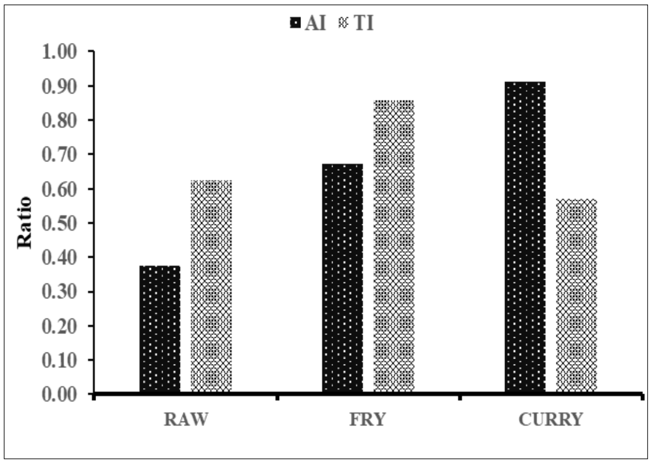
c. Indian mackerel (*Rasterlliger kanagurata*)



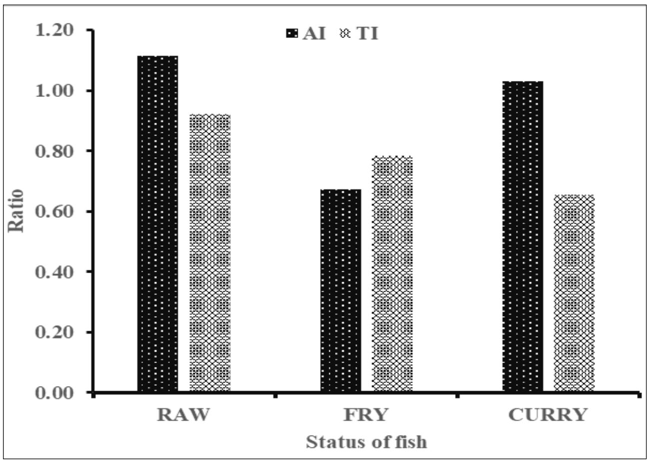
d. Wolf herring (*Chirocentrus dorab*)



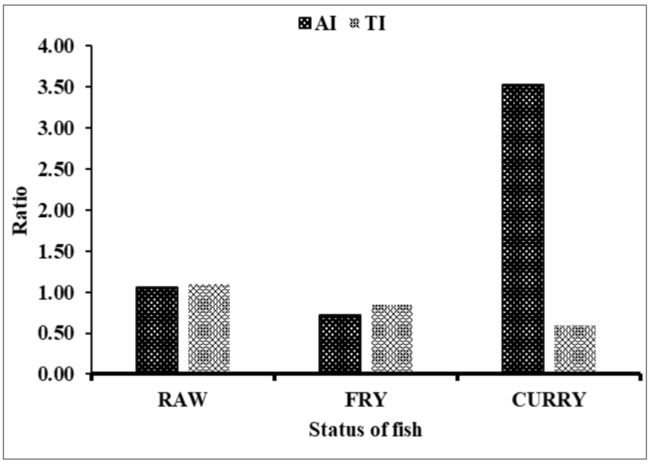
e. Mackerel scad (*Selar crumenophtthalamus*)



f. Skipjack tuna (*Katsuwonus pelamis*)



g. Barracuda (*Sphyraenea jello*)



h. Sardine herring (*Dussumieria acuta*)

Fig 2: Atherogenic (AI) and thrombogenic (TI) indices in raw, curried, and fried fish.

2. Fate of fatty acid composition in processed fish

All eight varieties of processed fish (Pony fish, mullet, Indian mackerel, wolf herrings, mackerel scad, tuna, barracuda, and sardine herrings) are commonly consumed fish species in Sri Lanka. All fish cooked as curry or fried showed a significantly high level of SFA owing to the presence of lauric acid (C12:0), myristic acid (C14:0) and palmitic acid (C16:0) in coconut milk and coconut oil, respectively (Table.3).

Tuna are major marine part of the Sri Lankan diet. Fresh tuna showed a total SFA content of 28.22 %, whereas cooked and fried tuna showed an SFA content of 37.36 % and 39.13 %, respectively (Table 4). The rise in SFA content in cooked and fried fish was due to the coconut cream and oil used for fish preparation. MUFA content also increased significantly from fresh (18.26 %) to fried (38.47 %) or curried (22.16 %) tuna. These results are in agreement with previously published work [22]. In contrast, PUFA content decreased significantly from fresh (45.13 %) to fried (17.22 %) or curried (32.91 %) tuna [22]. reported that SFA content decreases in fish after frying. In cooked tuna, the SFA content reduced by 70 % (from 15.5 % to 4.7 %), which is mainly due to reduction in the content of C14:0, C15:0, C16:0, C18:0, and C22:0 fatty acids. However, the results presented here showed an increase in SFA content during frying of tuna owing to the absorption of lauric acid and palmitic acid from coconut oil and cream.

The MUFA content in tuna increased during the frying stage owing to the increase in oleic acid content. The MUFA content also suffered a 45 % loss upon frying for 10 min. This was mainly owing to reduced content of C14:1, C15:1, and C24:1. The oleic acid (C18:1n9) content increased, which may be owing to their absorption from the frying palm oil. Decrease of PUFA content in tuna during cooking and frying was due to the loss of C18:3n6 and C20:5n3. The PUFA content increased 4.5 times in curried fish due to the absorption of C20:4n6, C22:6n6, and C22:6n3 from coconut milk. Major fatty acids such as C14:0, C16:1, and C22:6 in raw fish fillets are lost during frying (Candela *et al*., 1998). Among n-3 PUFAs, the essential fatty acids, the content of C18:3n3 (71 %), C20:3n3 (24 %), C20:5n3(10.8 %), C22:5n3 (28 %), and C22:6n3 (58.2 %) decreased in fried compared to fresh tuna fish, whereas in curry, the content of C18:3n3 (67 %) decreased and that of C20:3n:3 (39 %), C20:5n3 (18 %), C22:5n3 (18 %), and C22:6n3 (16.40 %) increased (Table.2).

The content of fatty acid C20:5n3 was reduced upon frying, whereas that of C22:6n3 remained more or less constant in fried and high in curried fish. The present results revealed a major loss in C18:3n3 content in fried (71 %) and curried (67 %) tuna, and [23] described a loss in C20:5n3 (69 %) and C22:5n3 (84 %) content in fried tuna compared to its raw counterpart. Therefore, fried fish is not a good source of n-3 fatty acids, although C18:3 is present, which was also suggested by. [24] showed that the cooking of tuna does not result in major destruction of omega-3 fatty acids (EPA and DHA), whereas microwave cooking results in a loss of 25 % of EPA (C20:5n3) and 55 % of DHA (C22:6n3). In addition, these authors showed that canning completely destroys these fatty acids, whereas frying results in about 70 % loss in EPA and 85 % loss in DHA contents. The results presented here show that, among n-6 PUFA, the content of C18:3n6 (53 %), C22:4n6 (33 %), C20:2n6 (9 %), C20:4n6 (13 %), and C22:5n6 (78 %) decreased in fried tuna, whereas that of C18:3n6 (54 %) decreased and that of C20:2n6 (2 %), C20:4n6 (33.4 %), C22:4n6 (5 %), and C22:5n6 (23.7 %) increased in curry. A high content of palmitic acid in all fish samples fried in coconut oil in fried fish. The MUFA content increased markedly in all fried fish samples owing to the high content of oleic acid in coconut oil (Table.2). Among PUFA, the content of omega-3 PUFA increased in mullet, Indian mackerel, wolf herring, and barracuda, and that of omega-6 increased in pony fish, mullet, wolf herring, mackerel scad, and tuna.

Table 3: Hypocholesterolaemia: hypercholesterolaemic fatty acid (HH) ratio in raw, fried, and curried fish

Fish name	Raw fish (HH)	Fried fish (HH)	Curried fish(HH)
Pony fish	0.68	1.3	0.7
Mullet	0.51	1.22	1.41
In. Mackerel	4.17	1.12	2.05
Wolf Herring	0.55	1.25	1.72
Mackerel scad	0.86	1.55	1.31
Tuna	1.32	1.29	1.84
Barracuda	0.96	1.56	1.33
Herrings	0.24	1.41	0.5
R value	-0.5	-0.45	0.64
P value	0.21	0.26	0.09

The EPA+DHA:C16:0 ratios are considered a good index to assess lipid oxidation [25]. Our data showed that fish made into curry (particularly, mackerel and barracuda) had higher lipid oxidation activities than fried samples. Overall, fish processed into curry had higher hypocholesterolaemic: hypercholesterolaemic (HH) ratios (Table 3). The HH ratio is useful in relating PUFA, considered hypocholesterolaemia (decrease in cholesterol levels), to the sum of two saturated fatty acids, namely, myristic acid (C14:0) and palmitic acid (C16:0), considered hypercholesterolaemia (increase in cholesterol levels) (Testi *et al*., 2006). Stearic acid (C18:0) should be excluded from the saturated fraction in this ratio because it does not behave like a typical saturated fatty acid in regard to affecting cholesterol levels [26]. showed that the HH value of raw trout is 2.65, which increases in microwaved and fried samples but decreases significantly (P<0.05) in boiled samples. Therefore, contrary to expectations, frying enhanced the nutritional value of trout from the point of view of increasing the HH ratio. The HH index was higher in all processed fish (fry and curry) compared to raw fish in these findings. [27] found higher values of HH in fish, varying from 2.03 to 2.46. The present study showed that the HH ratio in fried and curried fish ranged from 1.12 to 1.84 (Table 5), and revealed a decrease in cholesterol levels. The content of health beneficial FAs (EPA, DHA) decreased significantly by the traditional cooking methods of Asian tuna fish. By using coconut oil for frying and coconut cream for processing, the nutritional quality decreased compared to raw fish, whereas the content of health beneficial fatty acids and tocopherol was significantly lower in processed fish. A significant increase in energy content was observed in all cooked samples, whereas the cholesterol levels decreased. These results reveal that traditional cooking and frying method are not good method of fish consumption. But, microwaving is the best cooking method for a healthy consumption of rainbow trout [26].

4. Conclusion

The lipids in fish fillets play an important role in providing taste, flavour, smell, and texture to the fish. Our results show that fatty acid compositions changed significantly during fish preparation by either cooking as curry or frying in coconut oil, and resulted in lower nutritive values than raw fish. Particularly, the healthy lipids EPA and DHA were lost during fish preparation. The thrombogenic index was significantly increased in fried fish, adding to the evidence that consuming fried fish is less beneficial to human health than eating fish cooked in coconut cream.

No conflict of interest

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